# A NEW PROSPECT FOR CALIFORNIA BOTANY: INTEGRATING BIOSYSTEMATICS AND PHYLOGENETICS

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ABSTRACT

Integration of biosystematics (experimental study of biological aspects of organismal variation, diversity, and diversification) and phylogenetics (study of genealogical relationships of organisms) is a particularly promising avenue for future evolutionary and ecological investigations of the California flora. The exceptionally strong tradition of biosystematics in California botany has yielded findings that are responsible for much of our understanding of evolutionary processes in plants. The value of this research is, in part, attributable to a focus on the endemic plant lineages of California, which have provided ideal systems for investigating diverse modes of speciation and other evolutionary phenomena. An exciting new challenge to California botanists is reanalysis of biosystematic data and conclusions from a phylogenetic perspective. With understanding of phylogeny comes clarification of historical patterns and directionality of evolutionary changes and provision of more meaningful contexts for evolutionary comparisons. Phylogenetic research has indeed improved our understanding of speciation patterns, processes of diversification, and biogeographic relationships within California plant groups that were the subjects of earlier experimental studies. Only a small fraction of the California flora has been investigated from both biosystematic and phylogenetic perspectives.

Plant biosystematics, as defined here, is the experimental study of biological phenomena that are important for understanding plant variation, diversity, and diversification (see Grant 1984). Biosystematic studies include, for example, investigations of breeding systems, pollination biology, crossability and fertility relationships, chromosome evolution, niche relationships, and genetic and environmental components of phenotypic expression. In general, these types of studies involve some degree of experimental manipulation of living plants, such as crossing or transplanting. In contrast, phylogenetics is an analytical approach for reconstructing organismal genealogies (see Mishler this volume). Phylogenetic studies can be based on strictly descriptive data, usually from morphology or macromolecules; most systematic studies involving DNA sequences may be better classified as descriptive rather than biosystematic. It is important to note, however, that phylogenetics can be applied to, and is especially informed by, experimental data. DNA studies in the systematics community at large and in the Jepson Herbarium in particular extend, but do not replace, the tradition of descriptive

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research on morphology that remains a pillar of plant systematics and taxonomy. Molecular investigations, and phylogenetic studies in general, are new components of the "unending synthesis" in systematic botany (Constance 1964).

## The Importance of Biosystematics and Phylogenetics to California Botany

The richness of botanical diversity in herbaceous, particularly annual, groups amenable to in-depth experimental investigation has been a major factor in promoting biosystematic research in California. Most importantly, like other regions of the world with a Mediterranean climate, California contains an unusually high number of large, neoendemic lineages that are ideal, natural study systems for biosystematists interested in plant speciation and evolution. The ecological components of biosystematic research have proven especially critical to understanding California plant evolution. Extreme heterogeneity and dynamism of soils, climate, and topography in California have apparently been major stimuli to evolution in the flora, wherein diversification within plant lineages has often spanned highly contrasting environments (see Stebbins and Major 1965; Raven and Axelrod 1978).

An exceptional wealth of biosystematic data from many of our most characteristic groups of California plants has accumulated since the early part of this century. In fact, some of the first biosystematic studies undertaken in plants were those of such famous Californian botanists as Babcock, Hall, Stebbins, and the Clausen, Keck, and Hiesey team (e.g., Babcock and Hall 1924; Stebbins 1950; Clausen 1951). These pioneering scientists laid much of the foundation of biosystematics for an impressive succession of Californian plant researchers in the latter half of this century (reviewed in part by Raven and Axelrod 1978; Grant 1981).

In contrast to the strong tradition of biosystematics in California, few phylogenetic studies of California plants have been published. This lack of attention to phylogenetics in California botany is partly attributable to the recency of theoretical advances (Hennig 1966; see Mishler this volume) and technological innovations (see Hillis and Moritz 1990; Swofford 1993) that have made phylogenetic analysis feasible. Also, most plant phylogenetic studies have focused on groups that include economically important species (e.g., Palmer et al. 1983; Doyle et al. 1990; Wendel and Albert 1992) or on questions pertinent to understanding the broad-scale pattern of plant evolution and to refining higher-level classification (e.g., Jansen et al. 1990; Chase et al. 1993). Those phylogenetic studies that have addressed relationships within California plant lineages, however, have offered important new insights into evolution and biogeography of the flora.

A phylogenetic framework can greatly aid the interpretation of biosystematic data by offering insights into the directionality and sequence of changes in biological attributes (e.g., breeding systems, chromosome numbers or arrangements, edaphic restrictions), in some cases allowing unequivocal determination of ancestral and descendent character states (see Maddison and Maddison 1992). In addition, phylogenetics can clarify whether occurrences of a biological correlation in different species of a plant group, such as dioecy and fleshy propagules, have arisen repeatedly from another condition, and are therefore perhaps ecologically or developmentally significant, or have arisen once and are shared among species because of a shared common ancestry (e.g., Donoghue 1989).

Phylogenetic studies can also allow interpretation of unavoidably incomplete biosystematic data within a more comprehensive organismal context. For example, traditional cytogenetic investigations can be limited in taxonomic scope by certain biological obstacles (e.g., crossing barriers, failure of meiotic chromosomal association in hybrids, or hybrid inviability), but these limitations do not restrict the extent of species sampling in non-experimental phylogenetic studies. Phylogenetic results can thereby extend partial cytogenetic data by offering an expanded, directional perspective on chromosome evolution and the origin of breeding barriers within species lineages (e.g., Baldwin 1993, 1994).

## Examples from the California Flora

Phylogenetic studies can play a major role in advancing experimental research on the California flora by focusing biosystematic efforts on important, unforeseen relationships. One of the most prominent examples of this type comes from the phylogenetic work of Sytsma and Gottlieb (1986a, b) on *Clarkia* (Onagraceae). The genus *Clarkia* has been the subject of more extensive biosystematic investigation than any other genus of California plants. Research on *Clarkia,* primarily by Harlan Lewis and colleagues/students (e. g., Lewis 1973; Vasek and Weng 1989) and, more recently, by Les Gottlieb and associates (e.g., Gottlieb 1974, 1993), has generated a phenomenal amount of cytogenetic, isozymic, ecological, breeding system, and developmental data. Results from biosystematic investigations of *Clarkia* have, in turn, greatly influenced our understanding of plant evolution. Recent phylogenetic studies, based on chloroplast DNA (Sytsma and Gottlieb 1986a, b; Fig. 1) and nuclear ribosomal DNA sequences (Hahn et al. 1993), forced a rethinking of generic delimitations when the only species of *Heterogaura, H. heterandra,* was found to have been derived from within *Clarkia.* This finding prompted submergence of *Heterogaura* within *Clarkia* (Lewis and Raven 1992) and raised new questions about floral and

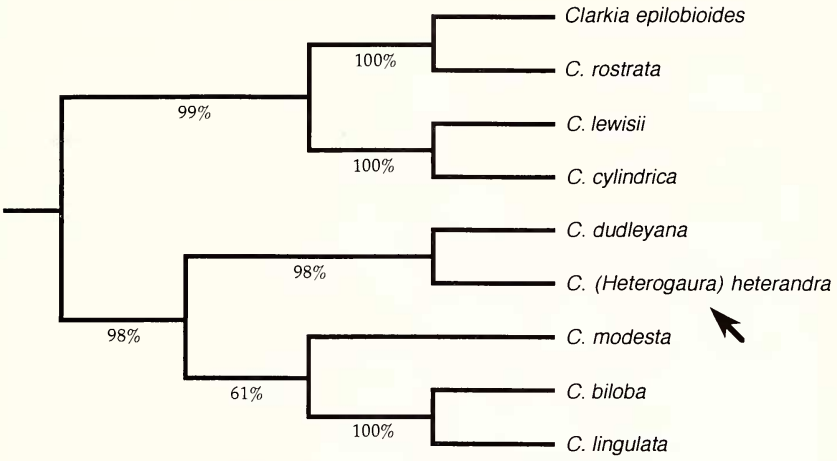


FIG. 1. Phylogenetic tree of *Clarkia* sect. *Peripetasma* (Onagraceae) based on chloroplast DNA restriction site mutations (redrawn from Sytsma and Gottlieb 1986a, b). Sytsma and Gottlieb reconstructed one minimum-length tree using Wagner parsimony, which was rooted with restriction site data from species of sect. *Phaeostoma* (*C. xantiana*) and sect. *Rhodanthos* (*C. amoena*). Percentages below branches are bootstrap values. Note the position of *C.* (=*Heterogaura*) *heterandra* (arrow).

fruit evolution in *Clarkia* that may be approached experimentally (e.g., how and why did the nut-like fruit of *C. heterandra* originate from the typical capsular fruit of *Clarkia*?). Discovery of the unexpected relationship of *C. heterandra* to other species of *Clarkia* offers a new avenue for expanded biosystematic and evolutionary research in the genus.

Another Californian example of phylogenetics serving to guide biosystematics is from the research of Baldwin and colleagues on the origin of the Hawaiian silversword alliance (*Argyroxiphium, Dubautia, Wilkesia*; Compositae). Carlquist (1959) demonstrated unequivocally on the basis of anatomical comparisons that the Hawaiian-endemic silversword alliance was most closely related to Madiinae, a primarily Californian group known as tarweeds or tarplants. Subsequent attempts to seek biosystematic evidence about the precise relationship of the Hawaiian species to the California tarplants was stymied by the inability to produce hybrids between members of the two groups (G. D. Carr and D. W. Kyhos personal communication). A chloroplast DNA phylogeny of the Californian and Hawaiian species refocused this biosystematic effort by suggesting that species of *Madia* and *Raillardiopsis* are the closest living relatives of the Hawaiian silversword alliance (Baldwin 1989; Baldwin et al. 1991), a result corroborated by later phylogenetic analysis of nuclear ribosomal DNA sequences (Baldwin 1992; Fig. 2). In ad-

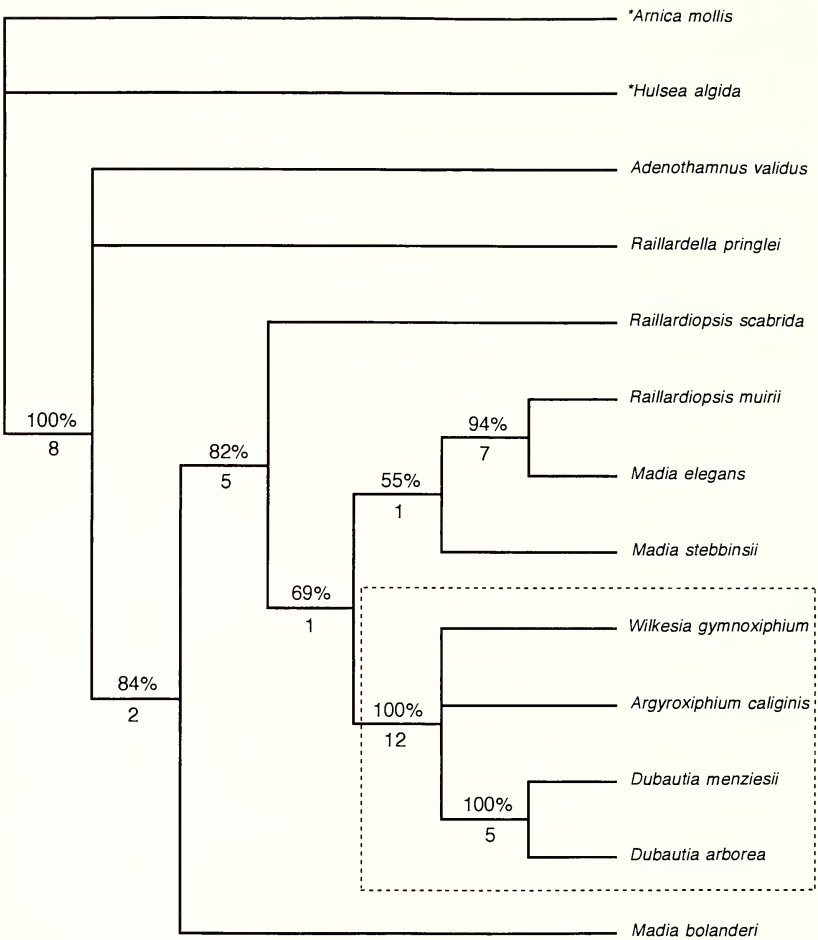


FIG. 2. Phylogenetic tree of select species from Californian and Hawaiian Madiinae (Compositae) based on internal transcribed spacer sequences of nuclear ribosomal DNA (modified from Baldwin 1992). This phylogeny is the strict consensus of the six minimum-length trees reconstructed using Fitch parsimony. Asterisks denote outgroup species. Percentages above branches are bootstrap values. Numbers below branches are decay index values. Dashed box surrounds the lineage of Hawaiian silversword alliance species. Note that the Hawaiian lineage is derived from within a grade of California tarplant species in *Madia* and *Raillardiopsis.* A similar pattern was reconstructed by Wagner parsimony analysis of chloroplast DNA restriction site mutations (Baldwin 1989; Baldwin et al. 1991).

dition, the DNA data demonstrated that *Raillardiopsis muirii* and *R. scabrida,* previously included within *Raillardella,* are most closely related to *Madia* and the Hawaiian silversword alliance. New attempts to create hybrids between the Californian and Hawaiian groups and between *Madia* and *Raillardiopsis,* guided by knowledge

of phylogenetic relationships, were successful (Baldwin 1989; Kyhos et al. 1990; Baldwin et al. 1991).

Recognition that the Hawaiian silversword alliance originated from within a sublineage of Californian Madiinae highlights the need for Californian botanists to keep a broad geographic perspective about possible relationships of even the most narrowly endemic plants in California. Another example that reinforces this caution is from work by Crawford and colleagues on *Coreopsis* (Compositae). Well-supported phylogenetic relationships of chloroplast DNA in *Coreopsis* (Compositae) suggest that the Californian annual species, previously considered to comprise a single lineage, may not be a natural (i.e., monophyletic or even paraphyletic) group (Crawford et al. 1991). Instead, the chloroplast DNA tree suggests that five of these six annuals are more closely related to the mainland Mexican perennials, *C. cyclocarpa* and *C. mutica,* and the Californian maritime perennials, *C. gigantea* and *C. maritima,* than to the remaining Californian annual, *C. stillmanii.* Relationships among these species are the subjects of continuing investigation by Crawford.

Despite the widespread perception that phylogenetics cannot be applied to groups with a history of hybridization, phylogenetic analysis can, in fact, serve to test biosystematic hypotheses of introgression or reticulation, in part by taking advantage of the different modes of inheritance of nuclear and organellar genes. The genus *Helianthus* (Compositae) provides an important example of this type from the California flora. Rieseberg et al. (1988) used phylogenetic analysis to reanalyze reported introgression between *H. annuus* and *H. bolanderi* in northern California. According to the classic hypothesis of Heiser (1949), introgression of genetic material from *Helianthus annuus* into the serpentine race of *H. bolanderi* ("exilis") gave rise to the ruderal form of *H. bolanderi* ("weedy"). If this hypothesis is true, ruderal *H. bolanderi* should possess a subset of the biparentally-inherited nuclear markers of both parents and one of the uniparentally-inherited chloroplast DNA genomes of the parents. In fact, Rieseberg et al. (1988; Fig. 3) found that ruderal *H. bolanderi* possessed four unique chloroplast DNA and nuclear DNA markers that were not found in either of the presumed parental species. Phylogenetic analysis showed that these markers were best interpreted as mutations that had arisen following divergence of ruderal *H. bolanderi* from a common ancestor with serpentine *H. bolanderi.* Furthermore, all sampled individuals of serpentine *H. bolanderi* and *H. annuus* possessed chloroplast DNA markers that were absent in ruderal *H. bolanderi* and had apparently arisen since these entities shared a common ancestor with ruderal *H. bolanderi.* These data demonstrated that ruderal *H. bolanderi* is the sole representative of an ancient lineage rather than a recent product of introgressive hybridization.

Phylogenetic studies have also helped to advance our understand-

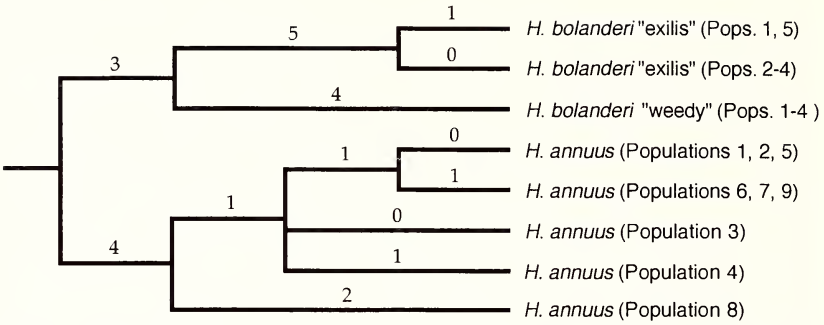


FIG. 3. Phylogenetic tree of 18 populations of *Helianthus annuus* and *H. bolanderi* (Compositae) based on chloroplast DNA and nuclear ribosomal DNA restriction site mutations (redrawn from Rieseberg et al., 1988). Rieseberg et al. reconstructed one minimum-length tree using Wagner parsimony, which was rooted with restriction site data from *H. maximiliani*. Numbers of restriction site mutations appear above branches. Note the four restriction site mutations that distinguish *H. bolanderi* "weedy" from *H. annuus* and *H. bolanderi* "exilis".

ing from biosystematics of the origin of hybrid and polyploid species in California. In *Microseris* (Compositae), Jansen and coworkers reexamined the origins of the Californian allotetraploids *M. decipiens* and *M. heterocarpa* from the perspective of a chloroplast DNA phylogeny and nuclear ribosomal DNA markers. They found that *M.* (*Uropappus*) *lindleyi,* suggested by biosystematic results to be one parent of the allotetraploids (see Stebbins et al., 1953; Chambers 1955), was actually more closely related to all members of *Agoseris* and *Nothocalais* than to *Microseris* sensu stricto, which includes the other putative, maternal parents of *M. decipiens* and *M. heterocarpa* (Jansen et al. 1991). These findings indicated that the hybridization events involved in the origins of the allotetraploids were between more distantly related species than had been appreciated previously. In *Raillardella* (Compositae), a genus of three primarily Californian, montane tarplant species, origin of the polyploid *R. scaposa* ($n$ = 34, 35) was unclear from cytological analysis of synthetic hybrids with *R. pringlei* ($n$ = 17), which possesses the same genomic arrangement as *R. argentea* ($n$ = 17) (Baldwin 1989; see Kyhos et al. 1990). Subsequent phylogenetic analysis of nuclear ribosomal DNA sequences from the three species indicates that *R. scaposa* is an allopolyploid involving species similar or identical to *R. argentea* and *R. pringlei.*

Phylogenetic studies can also help to distinguish among polyploid entities that have arisen independently but are morphologically and chromosomally similar. In *Microseris,* the chloroplast DNA tree of Wallace and Jansen (1990) provided evidence that "*M. heterocarpa*"

may be a polyphyletic species that includes populations that arose from at least two independent hybridization events between *M. lindleyi* and different annual taxa in *Microseris* (possibly different subspecies of *M. douglasii*). This observation, of course, calls into question the naturalness of this apparently polyphyletic species. In *Heuchera* (Saxifragaceae), Soltis et al. (1989) provided phylogenetic evidence from chloroplast DNA that indicates multiple origins of autopolyploidy within *H. micrantha,* a species that includes diploid and polyploid populations, in northern California and the Pacific Northwest. Three origins of autopolyploidy were inferred within one variety (*H. m.* var. *diversifolia*) alone.

Our understanding of diploid chromosome evolution in the California flora can also benefit from a phylogenetic perspective. Such cytogenetic clarification was obtained in *Calycadenia* (Compositae), a Californian genus of tarplants in which extreme chromosomal repatterning has occurred. Elegant cytological work on these species by G. D. Carr and R. L. Carr resolved cytological relationships in much of *Calycadenia* (see Carr 1975a, b; Carr and Carr 1983). Extensive chromosomal structural divergence of some species and lack of chromosomal association at meiosis in some hybrids, however, prevented comprehensive cytogenetic analysis (see Carr 1977). A highly-resolved and well-supported phylogeny of *Calycadenia,* based on nuclear ribosomal DNA sequences, extends understanding of evolution in chromosome numbers and chromosomal arrangements within the genus (Baldwin 1993; Fig. 4).

Baldwin's ribosomal DNA tree (Fig. 4) indicates that the only species of *Calycadenia* with a chromosome number of $n=9$, an absence of tack-glands, and an extreme southern California distribution, *C. tenella,* can be justifiably treated as a monotypic genus, *Osmadenia* (because *O. tenella* is the sister group of *Calycadenia*), in corroboration of Carr's (1975a) conclusions. The ribosomal DNA tree also supports Carr's hypothesis (1975a) that chromosome number differences in *Calycadenia* sensu stricto arose by descending dysploidy from a base number of $n=7$. The phylogeny extends the cytogenetic perspective by showing that two independent dysploid reductions in chromosome number from $n=7$ occurred in genus: one that gave rise to all species with $n=6$ and 5, and another that resulted in the only species with $n=4$, *C. spicata.* Further, the phylogenetic relationship of *C. hooveri* and *C. villosa* and their near-identity in chromosome arrangement (Carr 1975b) offers an insight into the actual chromosome arrangement possessed by the immediate ancestor of both sister dysploid lineages. Based on the combined perspective of these chromosomal and phylogenetic data, the ancestor of all species with $n=4$, 5, or 6 possessed a $n=7$ genome similar or identical in structure to that of either *C. hooveri* or *C. villosa.* Recognition that *C. hooveri* and *C. villosa* preserve (near-)

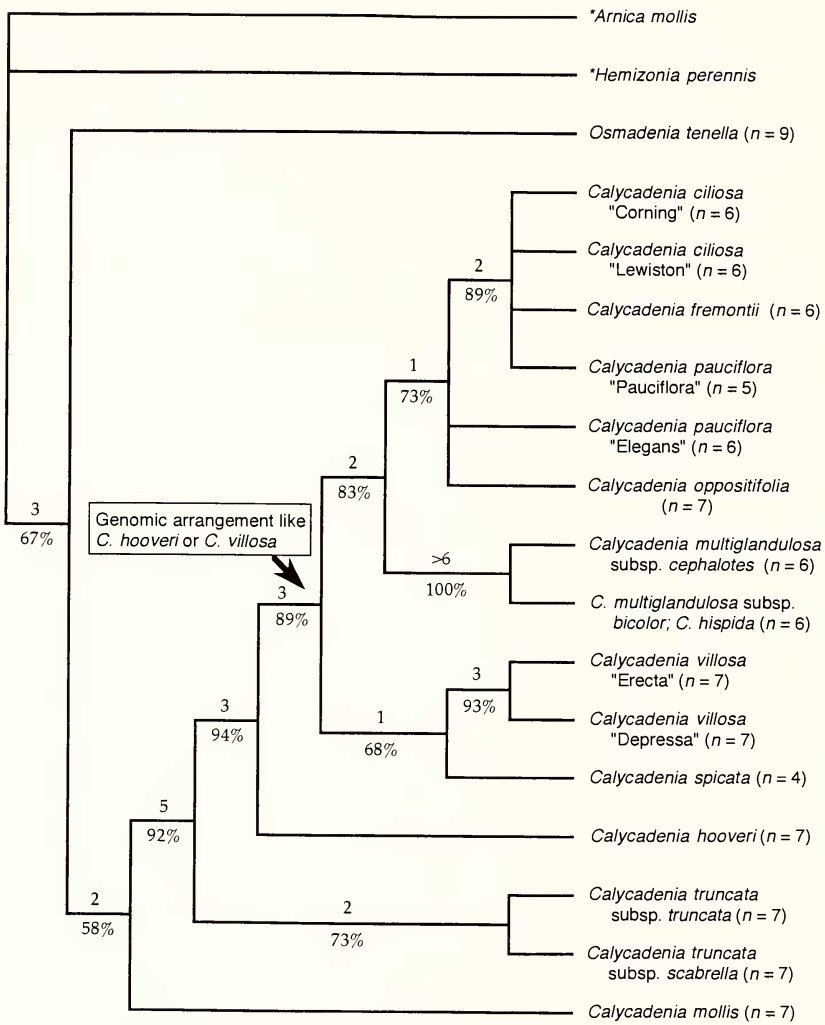


FIG. 4. Phylogenetic tree of *Calycadenia* (Compositae) based on internal transcribed spacer sequences of nuclear ribosomal DNA (modified from Baldwin 1993). This phylogeny is the strict consensus of the 11 maximally parsimonious trees reconstructed using Fitch parsimony. Asterisks denote outgroup species. Bootstrap values appear below branches. Numbers above branches are decay index values. Haploid chromosome numbers follow species names. Note the phylogenetic positions of *C. hooveri* and *C. villosa,* which share similar chromosome arrangements (Carr 1975b), and the consequent implication for the ancestral genome of both dysploid lineages (arrow).

relict chromosomal arrangements that may be of pivotal importance to understanding chromosome evolution in *Calycadenia* increases the conservation priority of these two rare species (both on CNPS List 1B, Skinner and Pavlik 1994).

Prominent examples of speciation modes from the California flora, based largely on biosystematic data, can also be tested and further refined with phylogenetic information. For example, phylogenetic analysis of *Layia* (Compositae) has offered a new outlook on the classic geographic speciation model proposed for this genus by Clausen, Keck, and Hiesey (see Clausen 1951), elaborated upon by Stebbins (1966), and further studied by Warwick and Gottlieb (1985). Clausen (1951) showed that in *Layia* highly interfertile species are allopatric, or effectively so, whereas truly sympatric species are of low interfertility or are apparently cross-incompatible. From these considerations (and secondary morphological criteria), it was concluded that speciation in *Layia* occurred during periods of geographic separation between gradually diverging populations, with sympatry arising after the development of reproductive barriers. This conclusion was based on the assumption that levels of fertility and chromosomal association at meiosis in hybrids were indicative of recency of common ancestry of the parental species. This presupposition violates the concept that derived characteristics diagnose relationships and further assumes that species interfertility and chromosomal homology (as reflected by extent of meiotic chromosomal association) decay gradually at similar rates throughout lineages. Violation of these assumptions in *Layia* could significantly alter inferred species relationships and, in turn, reduce conformity with the geographic speciation model.

Preliminary phylogenetic results from studies of nuclear ribosomal DNA sequences (Baldwin 1994, in prep.) suggest that species relationships are largely in accord with Clausen's assumptions: species that are highly interfertile and allopatric (e.g., *L. jonesii* and *L. munzii*) indeed appear to share a more recent common ancestry than species that are widely sympatric and of low interfertility (e.g., *L. chrysanthemoides* and *L. platyglossa*). In addition, extensive ribosomal DNA sequence divergence between species parallels high genetic divergence between species in allozymes (Warwick and Gottlieb 1985), thereby reinforcing Warwick and Gottlieb's (1985) conclusion that genetic evidence is consistent with gradual divergence of *Layia* species. Clausen's assumptions do appear to be violated by *L. carnosa,* however, which appears to be most closely related to species with which it is reportedly either intersterile or cross-incompatible, *L. gaillardioides* and *L. hieracioides.* In contrast, *L. pentachaeta* is partially interfertile with *L. gaillardioides* and *L. hieracioides,* but apparently more distantly related to these taxa than is *L. carnosa.* These unanticipated relationships, suggested by the ribo-

somal DNA phylogeny, are also among the best supported results from phylogenetic analysis of Clausen's morphological data matrix of *Layia* (Clausen 1951; Baldwin, in prep.).

The foregoing examples illustrate a few of the potential uses of phylogenetic information in conjunction with biosystematic data to advance our understanding of California plant diversity and diversification. From a practical standpoint, the importance of such evidence for taxonomy, floristics, and conservation efforts cannot be overemphasized. Realistically, we rely on taxonomy to reflect the natural lineages of life that are recognized as worthy of concern and protection. In turn, taxonomy must rely on phylogenetics and other systematic research to discern those critical lineages. In addition, floristic studies depend on a natural taxonomy for accurate estimates of biodiversity and as a basis for meaningful comparisons within and between bioregions. The California flora is sufficiently complicated and endangered to demand such detailed study in order to wisely set conservation priorities and to insure that limited conservation resources are used judiciously.

Biosystematic and phylogenetic studies are important components of the education and research program in plant systematics, conservation, and floristics at the Jepson Herbarium. The ability of the Jepson Herbarium to promote all types of California botanical studies, including biosystematic and phylogenetic investigations, has been greatly enhanced by the generosity of the previous Curator, the late Dr. Lawrence R. Heckard. Dr. Heckard's influence will continue to be felt at the Jepson Herbarium in many ways, including the Heckard Fund, established by Larry for continued research on the California flora into posterity.

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